Direct Measurement of Torque in an Optical Trap and Its Application to Double-Strand DNA

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We present a method that offers the possibility to directly apply and measure torque on particles in an optical trap. It can be used to rotationally manipulate biopolymers attached to appropriate particles. A flat object is trapped and oriented in the focus of a linearly polarized laser light. The direction and power of the orientational trap are controlled by the polarization state of the light. As a demonstration of the capabilities of the method, we examined the torsional stiffness of dsDNA (λ-DNA) in its linear torsional regime by directly measuring the torque generated by the molecule.

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During about the past decade, many important physical features of biological molecules (mainly polymers such as DNA, actin, and titin) have been explored based on single-molecule micromechanical experiments [1–17]. Researchers were able to manipulate and probe molecules individually, providing insight unimaginable before with traditional bulk measurements. Many of the investigations involve the use of optical tweezers, a versatile single-particle manipulation tool. In this technique, an optical trap is formed to hold a test particle in the micron size range with the molecule under investigation (e.g., DNA) attached to it. The other end of the molecule is typically linked either to another particle held by a micropipette or to a fixed surface (e.g., a glass cover slide). This way, it is possible to apply tension to the molecule and measure extension and force simultaneously, providing data to characterize the basic elastic features of the molecule. An impressive extension to this method has been demonstrated in a DNA manipulation experiment by the Bustamante group [16], where torsional strain could be generated and the emerging torque could be calculated while maintaining the feature of tension control. To achieve this, a third bead (biochemically linked to the molecule), a rotating micropipette, and fluid flow were necessary. Another method uses a magnetic trap to twist DNA by applying torque via a rotating magnetic particle attached to the molecule [11,13]. However, this torque cannot be measured directly. In this system, the tension can be controlled, too (extension and force can be monitored), and important data have been collected by measuring the extension of DNA as a function of turns of the molecule at different fixed tensions.

In this Letter, we discuss an optical trapping method that provides the opportunity to exert and measure torque directly on the manipulated molecule and demonstrate its usability in the case of dsDNA. In our system, the molecule is attached to the trapped object (a disk-shaped plastic particle of approximately 2 microns diameter) by one end and to a fixed plastic surface by the other end. The linearly polarized trapping light has an orienting effect on the flat test particle [18–21]. By rotating the plane of polarization, the orientation of the disk can be rotated; thus, the attached molecule can be twisted and torsionally stressed. As turns are added, the orientation of the disk drops behind the plane of polarization, because the torsional strain of the molecule acts against the orienting power of the light. By measuring the relevant angles (using video analysis), the molecular torsional stiffness can be directly compared to the orientational trapping power of the light. After calibrating the orientational trap, the torsional modulus can be determined.

Several possibilities have been introduced for orienting and rotating objects in optical tweezers. One approach uses birefringent particles in combination with linearly or circularly polarized light [22–24]; other arrangements utilize anisotropically shaped test particles and a trapping light beam with an anisotropic cross section [25]. Our method, i.e., controlling the orientation by the polarization state of light, offers a flexible and technically convenient way to extend an existing laser tweezers setup to include torque generation and measurement.

We used λ-DNA in its linear form of 15.6 μm contour length (Fermentas). Polystyrene microspheres of 1 μm diameter were squeezed mechanically to form disks. This was achieved by pipetting 1 μL of the original solution (Polysciences Polybead Polysterene Microspheres, 1 μm) between two laser window glasses (Casix, high precision BK7 windows) and applying a force in the range of 10^5 N for a few seconds in a hydraulic press. The disks were recovered from the glass surfaces by washing with distilled water, and by centrifugation a suspension with a particle density comparable to the original microsphere suspension was created. A 2-(N-morpholino)ethanesulphonic acid (MES) puffer of 50 mM, *p*H 5.0 was used in the experiments. 2 μL of the λ-DNA solution and 5 μL of the "disk suspension" has been added to 100 μL MES. This mixture was pipetted between two cover slips separated by a distance of 150 μm for an incubation time of several hours. The lower cover slip had a thin plastic layer on it created prior to incubation by spin coating a polysterene-toluene (50 mg/ml) droplet on the glass at 3000 rpm for 30 seconds

[26]. During the incubation period, the DNA molecules attached with certain probability to the plastic layer of the cover slip and/or to the disks [26]. Ultimately, the sample contained a significant number (approximately 10%) of disks that were connected to the surface via one DNA molecule. The probability that a disk is linked to the surface by more DNA molecules is negligible at these concentrations.

The optical tweezers is built around a Zeiss Axiovert 135 inverted microscope with a Zeiss Plan Apochromat oil immersion $100 \times /1,4$ objective. The optical tweezers was a Cell Robotics 980–1000 unit containing a single mode infrared diode laser SDL 5762 A6 working at 995 nm. Before reaching the objective, the trapping light passed through a special device responsible for controlling the orientational trap. This device contains a $\lambda/2$ and a $\lambda/4$ plate both driven by computer-controlled stepper motors. By rotating the $\lambda/2$ plate, the plane of polarization and, thus, the direction of the orientational trap can be rotated. By rotating the $\lambda/4$ plate, the polarization state can be changed between linearly polarized and circularly polarized; thus, the orientational power can be adjusted. The measurements were recorded with a video camera and transferred to a computer where image analysis was performed to determine the orientation of the trapped object in each movie frame (using the image and video measurement software iTraceLab).

The disk is trapped in the laser focus both in a translational and orientational sense. The object has an equilibrium position in the translational trap around which Brownian fluctuations can be observed. Similarly, it has an equilibrium angle in the orientational trap (defined by the polarization plane of the trapping light) around which rotational Brownian motion can be observed. In our case, the power of the translational trap is high compared to the orientational trap, so the translational Brownian fluctuations can be neglected, and the object can be regarded as rotating around the fixed optical axis. For linear traps, the torque τ acting on the object is proportional to the angle α measured between its actual and equilibrium orientation. According to the Boltzmann distribution, the probability density function of α is Gaussian:

$$\rho(\alpha) \propto e^{-(E/k_B T)} = e^{-(k\alpha^2/2k_B T)}, \tag{1}$$

where k is the torsional spring constant of the trap, T is the temperature, and k_B is Boltzmann's constant. By monitoring α over a sufficiently long period of time, the density function of α can be determined and fitted with a Gaussian; thus, k can be determined. This way the orientational trap can be calibrated.

If a molecular strand links the disk to the cover slip with nonrotating bonds (Fig. 1), an additional orientational trap is formed by the torsional strain of the molecule. In this case, the orientation of the trapped object will fluctuate around a new equilibrium orientation (EQ) determined by both the direction corresponding to the zero torsional strain of the molecule (M) and the polarization plane of the trapping light (P). In this equilibrium state (EQ), the torques generated by the molecule and the light are equal:

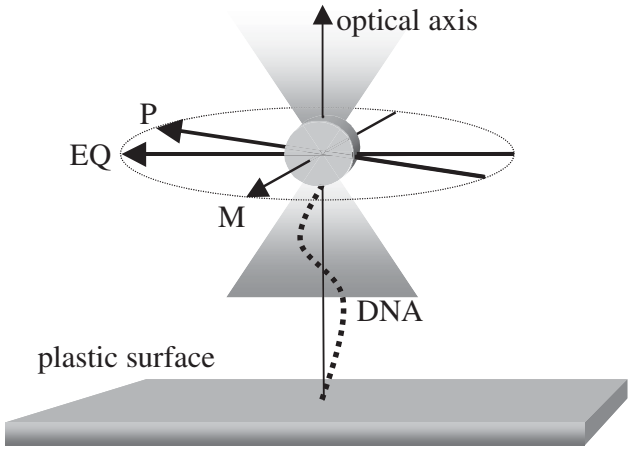

FIG. 1. Optical trapping arrangement. Notations: P, polarization plane of the trapping light; M, direction corresponding to the zero torsional strain of the molecule; EQ, equilibrium orientation of the disk.

$$k_M \alpha_M = k_L \alpha_L, \tag{2}$$

where k_M and k_L are the torsional spring constant of the molecule and the optical trap; the α_M, α_L angles are measured between EQ and M or P, respectively. Equation (2) determines the angular position of EQ according to the ratio of the torsional spring constants. If both orientational traps are working in the linear regime, the effective torsional spring constant k_{eff} of this combined trap will be

$$k_{\text{eff}} = k_M + k_L. \tag{3}$$

k_{eff} can be obtained by analyzing the rotational Brownian motion of the trapped object [using Eq. (1)]. By combining Eqs. (2) and (3), k_M could be obtained if we were able to measure the α_M and α_L angles. However, α_M cannot be determined easily, and it is easier to measure angle changes than absolute values. If we rotate the plane of polarization (P) by Δ_P, the equilibrium state of the trapped object (EQ) shifts by Δ_{EQ} (cumulative angle). Using Eqs. (2) and (3), the following expression can be derived for k_M:

$$k_M = k_{\text{eff}}\left(1 - \frac{\Delta_{\text{EQ}}}{\Delta_P}\right). \tag{4}$$

Finally, the torsional modulus G of the molecule is

$$G = k_M l, \tag{5}$$

where l is the (contour) length of the molecule.

The method used for linking DNA to the plastic surface and disk incorporates a nonspecific interaction, meaning that the molecule was attached at arbitrary (generally

multiple) positions to both plastic objects [26]. By evaluating measurement data, it was clear that, in most cases, the molecule could not freely rotate at its bonds. Those rare cases where no torque could be detected were rejected from the statistics. In the case of dsDNA, the torsional spring constant of the molecule is 3 orders of magnitude smaller than that of the light in our system. To measure this small effect, we twisted the DNA molecule several times in positive and negative directions symmetrically. In the experiments, we always stayed in the linear torsional regime of the polymer [16]; consequently, it was irrelevant whether the center position of the rotation protocol was a true equilibrium orientation of the polymer. The measurement proceeded as follows: The plane of the polarization of the trapping light and, thus, the disk was rotated by several turns in one direction with a relatively high speed (180° / sec) at maximum laser power. After this, the power of the trapping light was reduced to a level where the Brownian fluctuations of the disk became detectable. Here we started to rotate the plane of polarization at a lower speed (3.6° / sec) and added an additional two turns. From the data (angle of orientation of the disk) recorded during these two turns (data collection period), we calculated the equilibrium orientation of the disk and analyzed the Brownian fluctuations in order to determine the effective torsional spring constant k_{eff} (Fig. 2). Following this, the laser power was set again to maximum, the polarization was rotated back to its original state, and the same procedure was repeated in the opposite direction.

Knowing the shift of the equilibrium angle measured at the two twist extrema Δ_{EQ}, the change of the polarization Δ_P and k_{eff}, the torsional modulus of the molecule could be calculated using Eqs. (4) and (5). Typically, for 100 turns of Δ_P, the change of the relative orientation of the polarization and the disk $\Delta_P - \Delta_{\mathrm{EQ}}$ was about 3°. For each molecule, the above measuring procedure was performed at two relative extensions of 0.75 and 0.5. Note that each measurement had its own calibration (determination of k_{eff}); consequently, a possible variation of disk size, shape, or laser intensity had no effect on the accuracy. Also note that k_M can be determined, of course, without any additional rotation at the twist extrema by simply analyzing the orientational fluctuations of the disk at fixed polarization. However, rotating the polarization is an efficient way to reduce the effect of slight anisotropic errors present in the measuring apparatus (represented by the low frequency periodic component of the orientational angle during slow rotation, seen in Fig. 2).

In their statistical mechanics model of twist-storing polymers, Moroz and Nelson showed that the effective torsional modulus (and so the C_{eff} effective twist persistence length) of the molecule depends on its (fixed) relative extension [27,28]. The less the extension (pulling force) of the chain is, the stronger the thermal bend fluctuations are, resulting in a lower measurable torsional stiffness. The effect has been calculated quantitatively and shows a dependence on the ratio of the twist and bend persistence lengths C/A and the temperature. The smaller the bend persistence length A compared to the twist persistence length C is, the more reduced the measurable (effective) persistence length of the molecule is. At the maximum relative extension (when no bending is allowed), C_{eff} is equal to C and characterizes the microscopic (local) twist stiffness of the molecule.

Figure 3 shows the values of the effective torsional modulus measured at 0.5 and 0.75 relative extensions (8 data points, 95% C.I.) along with a fit to the Moroz-Nelson model yielding a value of 420 ± 43 pN nm^2 for the local torsional modulus of dsDNA (equivalent to a twist persistence length C of 102 ± 10 nm, using a value of 50 nm for A [29]).

In this Letter, we introduced a single-molecule manipulation method capable of applying and measuring torque directly on the investigated object. The method is based on

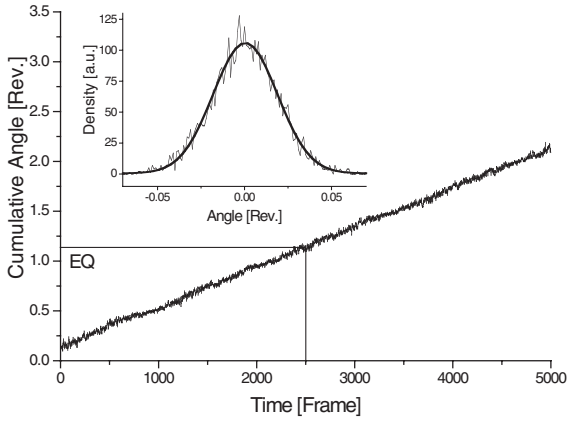

FIG. 2. Typical raw data showing the orientation of the disk during data collection at one twist extremum. The average of cumulative angles defines the equilibrium orientation EQ in the middle of the period. Calibration is based on the analysis of Brownian fluctuations (inset).

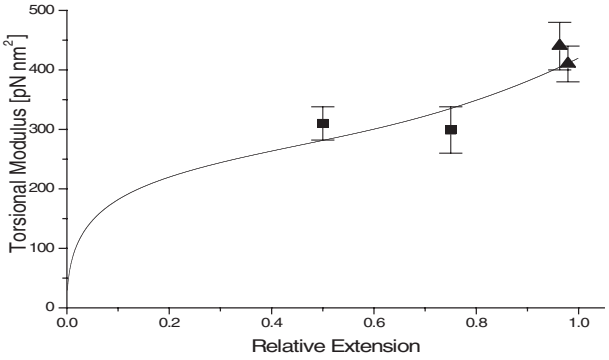

FIG. 3. Torsional modulus of dsDNA as a function of the relative extension: ■, experimental values; ▲, torsional modulus measured in Ref. [16] at 15 and 45 pN tensions and represented by the model in Refs. [28,30] fitted to our experimental values (solid line).

controlling the position and orientation of an anisotropic particle in an optical trap of linearly polarized light with the molecule attached to it and to a fixed surface with nonrotating bonds. By monitoring the orientation, the torsional spring constant of the molecule can be directly compared to the torsional spring constant of the optical trap. The measurement data themselves provide the source for calibrating the trap. The tension and torque applied to the molecule can be independently adjusted in a relevant range for potential biological investigations. The method is not equivalent to others also used successfully to torsionally manipulate biopolymers [13,16]; it offers the control of a different parameter set. The main properties of our method that differ from the others are (i) we can measure the torque upon the flat test particle directly; this also enables the application of a known torque upon the experimental object. (ii) The translational position of the test particle is well controlled in the optical tweezers; consequently, torsional manipulation can be performed at different extensions of the biopolymer. We believe these properties make it a useful addition to the experimental repertoire for torsional manipulation. We note that the method introduced recently where birefringent microscopic particles are grabbed and oriented in laser tweezers formed by polarized light to exert and measure torque [24] could, in principle, be used to achieve the same goal as presented here. From the point of view of the above listed characteristics, they are equivalent. Differences may be due to the technical realization, i.e., ease and accuracy of torque measurement, the applicability of birefringent particles to be attached to biomolecules, etc.

We described how the method can be used to calculate the torsional stiffness of a polymer in general. In the case of dsDNA, we demonstrated the method in action by probing the effective twist stiffness of the polymer at 0.75 and 0.5 relative extensions. Using the model of Moroz and Nelson, we obtained a value for the torsional modulus basically identical to that obtained recently by the Bustamante group (440 ± 40 and 410 ± 30 pN nm^2 measured at 15 and 45 pN tensions, respectively [16]). This value is significantly higher than those reported earlier from supercoiled single-molecule DNA stretching experiments using magnetic tweezers [11,13]. We note that those experiments were also evaluated by Moroz and Nelson, yielding a value of 109 nm for the local twist persistence length [27,28] in a remarkable agreement with recent experimental data ([16] and this work). When using the treatment of Moroz and Nelson for the magnetic tweezers experiments and our work, all available results are consistent, providing strong additional support for this model.

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